

## Environmental enteric dysfunction, soil transmitted helminthiasis and stunting among 36- to 59-month-old children in Quezon Province, Philippines

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### ABSTRACT

**Introduction:** Environmental enteric dysfunction (EED) is relevant in public health as it is a potential cause of child stunting. In the Philippines, stunting affects 33.4% of children in 2015. As of date, no local studies on EED exist. This study primarily aimed to determine the prevalence of EED, soil transmitted helminthiasis (STH) and stunting; and their relationships. **Methods:** A cross-sectional study was conducted among 120 children aged 36-59 months old in Quezon Province, Philippines. EED was assessed via glucose hydrogen breath test with a cut-off of  $\geq 20$  ppm over baseline by 90 minutes suggestive of small intestinal bacterial overgrowth (SIBO), a biomarker of EED. Kato Katz Technique was used to determine STH. Stunting was determined using the 2006 World Health Organization Child Growth Standards. Descriptive and correlation analyses of data were done at 10% level of significance. The study received approval from the National Ethics Committee of PCHRD-DOST, Philippines. **Results:** Data on expired hydrogen level of  $< 20$  ppm were documented. The prevalence of STH among children were 38.1% for *Ascaris lumbricoides*, 15.3% for *Trichuris trichiura*, and 1.7% for hookworm infections. A high rate of stunting at 40.0% was observed. There was a significant positive association between stunting and the presence of *Ascaris* ( $p=0.01$ ). **Conclusion:** There was no case of EED detected in the study. The severity of stunting was high at 40.0%. Among the STH, the prevalence of 38.1% children having *Ascaris lumbricoides* was alarming in terms of morbidity control. Nutrition interventions including WASH practices and the use of anthelmintic drugs need to be intensified to address stunting and STH.

**Keywords:** Environmental enteric dysfunction, stunting, small intestinal bacterial overgrowth, soil transmitted helminthiasis

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## INTRODUCTION

Stunting among children younger than 5 years is a major global health priority that has gained international attention because of its significant consequences on health and development. In the Philippines, stunting has remained unchanged over the years, affecting 33.4% children in 2015 (FNRI-DOST, 2016). Recognising that the primary drivers of undernutrition are inadequate dietary intake and recurrent infection, strategies to prevent and/or to address undernutrition are focused on dietary solutions, as well as water, sanitation and hygiene (WASH) interventions. However, both interventions have small to moderate impacts on linear growth. Dietary interventions impact linear growth from only 0.12 to 0.28 in terms of length-for-age Z-score (Dewey & Adu-Afarwah, 2008), while WASH interventions implemented with 99% coverage would reduce stunting prevalence by only 2.4% (Bhutta *et al.*, 2008). This indicates that the cause of stunting transcends beyond dietary inadequacy and infections. The pathogenesis of stunting is poorly understood (Owino *et al.*, 2016) and implicates that a subclinical disorder of the small intestine known as environmental enteropathy (EE) or environmental enteric dysfunction (EED) may have caused poor linear growth (Humphrey, 2009; Owino *et al.*, 2016).

EED is a condition when there are abnormalities in the intestinal mucosa and is characterised by the presence of bacterial overgrowth in the small intestine. Small intestinal bacterial overgrowth (SIBO) contributes to the pathogenesis of EED as overgrowth has been associated with EED domains such as intestinal inflammation and micronutrient malabsorption (Harper *et al.*, 2018; Donowitz *et al.*, 2016; Vonaesch *et al.*, 2018). EED is found at a

high prevalence among stunted children living under poor sanitary conditions and is pandemic in developing countries with limited resources (Owino *et al.*, 2016).

The impact of helminth colonisation on EED has not been well studied, but considering that the prevalence of intestinal soil transmitted helminthiasis (STH) infections is high, this study attempted to establish an association between STH and EED. STH infections remain a public health problem in many areas in the Philippines where challenges related to water, sanitation and hygiene continue to be experienced. Specifically, the prevalence of STH in the Philippines was noted at 27.7% for *A. lumbricoides*, 31.3% for *T. trichiura*, and 13.3% for hookworm (Soares Magalhães, 2015). The estimated prevalence of STH in the Philippines in school age children and preschool age children have not satisfied the targets of <20% cumulative prevalence and zero heavy intensity STH recommended by the World Health Organization (WHO) in order to achieve morbidity control (Belizario *et al.*, 2015).

As of date, no local studies are available that looked into the occurrence of EED in the Philippines. Hence, this study aimed to determine the prevalence of EED, STH and stunting among 36- to 59-month-old children; and their relationships. It also aimed to identify determinants of stunting among the study children.

## MATERIALS AND METHODS

A stratified multi-stage sampling was employed in this cross-sectional study among 36- to 59-month old children in the province of Quezon, Philippines, where stunting prevalence among children <5 years old was highest in the CALABARZON region at 33.9% (FNRI-DOST, 2016). This group of children was

selected because of their cognitive, social and motor skills that enabled them to comply with research protocols. All apparently healthy children who had no feeding problem and inborn/congenital/severe illness; who were not suffering from disease at the time of recruitment; who were not taking antibiotics for the last four weeks; residents in the barangay or municipality for the past six months; and with signed informed consent were included in the study.

To select the study areas, all municipalities in the Province were grouped into two strata, namely: low- and high-income municipalities. Per stratum, one municipality was randomly selected to represent the low (5<sup>th</sup> class) and high (1<sup>st</sup> class) income municipalities. Using the list of children 0-71 months old obtained from the Municipal Nutrition Action Officers, children aged 36-59 months from all qualified barangays were constructed as the sampling frame. The required sample size was estimated based on the prevalence of stunting (33.9%) among children in Quezon and the total number (124,411) of children aged 36-59 months in Quezon with a sampling error of 8%, level of confidence of 90%, and a design effect of 1.3. A total of 120 children was computed and were randomly drawn from the master list obtained from the Municipal Nutrition Action Officers. A randomly selected replacement was considered in case of disapproval from mothers to participate, decided to terminate participation in any stage of the study, or failed to conform with the preconditions for hydrogen breath testing.

The mothers or caregivers of the selected children were invited for a meeting to explain the nature and purpose of the study, as well as the study protocols. Assistance from the municipal and barangay officials, health and nutrition workers, and day-care teachers who were oriented on the study

were tapped to encourage participation of the respondents.

Glucose was used as the test substance for the diagnosis of SIBO using the Hydrogen Breath Test Sleuth by Breathe EZ Systems, Inc. In this study, EED was defined with SIBO as the biomarker for the pathophysiological changes of malabsorption and intestinal inflammation characterising EED. Study children were examined by a paediatrician before enrolment to ensure that they were physically/medically fit for the Glucose Hydrogen Breath Test. Demonstration on how to breath using disposable mouthpiece and how to release expired air to the breath analyser was also conducted by the municipal nurse or the researcher. Mothers/caregivers were advised not to give their children any slowly digested foods, any fibre supplements and laxatives for 24 hours prior to the test, and to fast for 8-12 hours before the procedure. On the day of assessment, mouth rinse was first administered to children in order to avoid the possibility of an early peak in hydrogen associated with the action of bacteria in the mouth on the carbohydrates used in the test. Children were instructed not to swallow the mouthwash. The test began with the collection of a basal expired air (fasting sample). Baseline breath hydrogen was measured by letting children take a deep breath of air and expired into the mouthpiece until all the air was out of the lungs. The presence of bacterial overgrowth was ruled out before performing the glucose breath test. Children who were eligible but with a baseline expiratory breath of >10 ppm, which is indicative of bacterial overgrowth, were excluded from the study. Glucose was then administered orally. A glucose load of 1.75g/kg body weight up to a maximum of 75g (Harriet Lane Handbook, 2017) dissolved in 10ml/kg body weight up to a maximum

of 250 ml (Eisenmann *et al.*, 2008) was appropriate for children. Using this as reference, the glucose drink (Medic orange, glucose test beverage) formulated by the Medic Diagnostic Laboratory in Pasig City, Philippines was used as the test substance. Samples of expired air were measured at 30-, 60- and 90 minutes after glucose load. The 2017 North American Consensus was adopted such that the length of glucose testing was limited to two hours; and the diagnostic criteria used was a rise in expired hydrogen of  $\geq 20$  ppm from baseline by 90 minutes as the ideal criterion for a positive test to suggest the presence of SIBO (Rezaie *et al.*, 2017).

Height measurements were carried out following the standard procedures and recommended equipment as prescribed in the National Health and Nutrition Examination Survey (NHANES) Anthropometry Procedures Manual (CDC, 2013). Microtoise was used to measure the height of children in centimeters. Two measurements of height were taken and recorded to the nearest 0.1cm. A third reading was required when the two measurements were beyond the acceptable limits (0.5cm for height). Age in months of each child was also collected to enable the determination of nutritional status. The corresponding Z-scores were computed using the 2006 WHO Child Growth Standards (CGS) software to determine the nutritional status of the children. Children with Z-scores  $< -2$  standard deviation (*SD*) for height-for-age were classified as stunted.

The collection, packaging, storage and transport of faecal samples followed the procedures prescribed by the Department of Parasitology, University of the Philippines, Manila, where parasitological assessment was done. Each mother/caregiver received an improvised paper triangle lined with wax paper instead of a plastic cup,

wooden stick and re-sealable plastic bag for one stool specimen of each child. A demonstration of stool collection was done in such a way that thumb-size stool was to be placed in the paper triangle using the wooden stick, then folding the paper to secure the specimen and putting it in a re-sealable plastic bag. The respondents were informed of the schedule of collection either at a specified venue or via house visits. Immediately after submission, sealed and labelled specimens were placed in an insulated box (styrofoam box) packed with ice and transported to the laboratory within 24 hours after collection. Parasitological assessment utilised the Kato-Katz technique following the WHO's Bench Aids for the Diagnosis of Intestinal Parasites (WHO, 1994). Microscopic reading of the slides was done by the microscopist to determine the prevalence of individual STH species, namely: *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm.

Data entry was done in Microsoft Excel and analysed using the Statistical Package for Social Sciences (SPSS) software version 23. Data cleaning for inconsistencies, errors, double entry were performed before data analysis.

The profile and distributional characteristics of the subjects were described using appropriate descriptive statistics and by constructing percent distributions. Correlation analysis was done to determine the significant associations between variables. In particular, point biserial correlation coefficient was used for correlating dichotomous variables with quantitative data, while Cramer's *V* coefficient (based on chi-square) was used for nominal variables. Significant factors were determined at 10% level of significance.

This research received ethical approval from the National Ethics Committee of the Philippine Council for Health Research and Development of the

**Table 1.** Distribution of nutritional status in children 36-59 months in Quezon Province, Philippines based on height-for-age Z-score (HAZ)

Nutritional status based on Height-for-age Z-score (HAZ) (n=120)	Magnitude (%)
Severely stunted (<-3SD)	7.5
Moderately stunted (<-2SD to -3SD)	32.5
Normal (-2SD to +2SD)	60.0

Department of Science and Technology (PCHRD-DOST), Philippines.

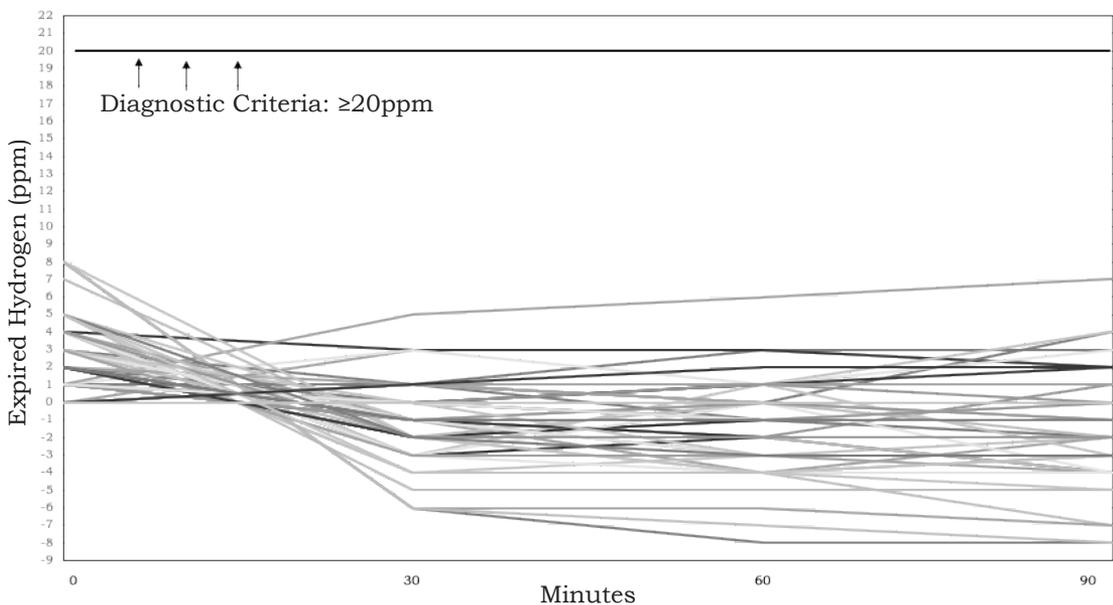
## RESULTS

Overall, the prevalence of stunting in the study area was 40.0%, with 7.5% children classified as severely stunted and 32.5% moderately stunted (Table 1). With reference to the WHO cut-off points (WHO, 1995), the severity of stunting in this study was very high at 40.0% magnitude. This prevalence was higher compared with the 2015 national prevalence of 33.4% for stunting, but consistent with Quezon Province at 40.0% based on the FNRI National

Nutrition Survey in 2015 (DOST-FNRI, 2016).

Results of the parasitological assessment revealed that *Ascaris lumbricoides* infections were seen in 38.1% of the study children; *Trichuris trichiura* infections were seen in 15.3% of the study children; and for hookworm infections, a prevalence of 1.7% was observed.

Figure 1 shows the expired hydrogen of 120 children from baseline and by 30-, 60-, and 90-minutes after ingestion of glucose. By definition, a positive test to suggest the presence of EED is a rise of  $\geq 20$ ppm from baseline in breath

**Figure 1.** Expired hydrogen of 120 children aged 36-59 months at baseline, 30-, 60-, and 90-minutes after ingestion of glucose

**Table 2.** Measures of the associations between stunting and socio-demographic-economic variables, food intake, feeding and health practices, WASH variables, and STH in children 36-59 months in Quezon Province, Philippines

Variables	Stunting	
	Correlation coefficient	p-value
Socio-demographic-economic variables		
Household size	0.114 <sup>a</sup>	0.22
Household income	0.118 <sup>a</sup>	0.20
Nutrient intake		
Energy	-0.088 <sup>a</sup>	0.34
Protein	-0.164 <sup>a</sup>	0.07*
Calcium	-0.176 <sup>a</sup>	0.05*
Phosphorus	-0.279 <sup>a</sup>	<0.001***
Iron	-0.093 <sup>a</sup>	0.31
Vitamin A	-0.198 <sup>a</sup>	0.03**
Riboflavin	-0.273 <sup>a</sup>	0.00***
Niacin	-0.025 <sup>a</sup>	0.77
Feeding and health practices		
Breastfeeding after birth	0.006 <sup>b</sup>	0.95
End of exclusive breastfeeding	0.325 <sup>b</sup>	<0.001***
Full immunisation	0.185 <sup>b</sup>	0.04**
WASH variables		
Type of drinking water	0.185 <sup>b</sup>	0.04**
Drinking water service level	0.292 <sup>b</sup>	0.01**
Toilet classification	0.170 <sup>b</sup>	0.06*
Toilet service level	0.181 <sup>b</sup>	0.14
Hand washing service level	0.022 <sup>b</sup>	0.81
Appropriate hand washing techniques	0.150 <sup>b</sup>	0.10
Soil transmitted helminthiasis		
Ascaris presence	0.252 <sup>b</sup>	0.01**
Trichuris presence	0.136 <sup>b</sup>	0.14
Hookworm presence	0.027 <sup>b</sup>	0.77

<sup>a</sup>correlation coefficient using Point Biserial

<sup>b</sup>correlation coefficient using Cramer's V

\*significant at  $p$ -value<0.10

\*\*significant at  $p$ -value<0.05

\*\*\*significant at  $p$ -value<0.01

hydrogen by 90 minutes (Rezaie *et al.*, 2017). In this study, the mean expired hydrogen concentration at 90 minutes from baseline was 0.73ppm (Range: 0,8). Results showed that children demonstrated no increase in breath hydrogen greater than the diagnostic criteria of 20ppm. Hence, there was no case of EED detected in this study.

Correlation analysis is presented in Table 2. Results showed that stunting was significantly and negatively associated with nutrient intakes of protein ( $p=0.07$ ), calcium ( $p=0.05$ ), phosphorous ( $p<0.001$ ), vitamin A ( $p<0.001$ ), and riboflavin ( $p=0.00$ ). Moreover, stunting was found to be significantly associated with the age of

**Table 3.** Summary of significantly associated factors with stunted or not stunted children aged 36-59 months in Quezon Province, Philippines.

Variables	Stunting	
	Not Stunted	Stunted
Nutrients <sup>†</sup> (Mean Percent EAR)		
Protein	220.6	197.0
Calcium	122.0	94.7
Phosphorus	150.1	119.4
Vitamin A	154.5	118.7
Riboflavin	228.2	163.5
End of exclusive breastfeeding <sup>‡</sup> (%)		
< 6 months	65.1	34.9
≥ 6 months	46.9	53.1
Immunisation <sup>‡</sup> (%)		
Fully immunised	62.7	37.3
Incomplete immunisation	30.0	70.0
Classification of drinking water <sup>‡</sup> (%)		
Improved	68.2	31.8
Unimproved	50.0	50.0
Drinking water service level <sup>‡</sup> (%)		
Basic	70.7	29.3
Unimproved	33.3	66.7
Toilet classification <sup>‡</sup> (%)		
Improved	61.7	20.0
Unimproved	38.3	80.0
Soil transmitted helminthiasis <sup>‡</sup> (%)		
Positive for <i>Ascaris</i>	44.4	55.6
Negative for <i>Ascaris</i>	69.9	30.1

<sup>†</sup>Comparison using *t*-test for independent samples

<sup>‡</sup>Comparison using chi-square test

child when exclusive breastfeeding was stopped ( $p < 0.001$ ), full immunisation ( $p = 0.04$ ), type of drinking water ( $p = 0.04$ ), drinking water service level ( $p = 0.01$ ), and toilet classification ( $p = 0.06$ ). With STH, there was a significant positive association between stunting and the presence of *Ascaris* ( $p = 0.01$ ).

The summary of significantly associated variables with stunting or no stunting is shown in Table 3. The mean estimated average requirement (EAR) for nutrients was higher in children who

were not stunted compared to those who were stunted. The proportion of children who were not stunted were higher for those who were exclusively breastfed just under six months (65.1%) and fully immunised (62.7%). Higher proportion of children who were not stunted used improved drinking water (68.2%), basic water service level (70.7%), improved toilet facilities (61.7%), and practised appropriate hand washing techniques (67.9%). For those who used unimproved toilet facilities, more children were

stunted (80.0%) than those who were not (38.3%). The percentage of children found positive for *Ascaris* and stunted was higher (55.6%) than those who were not stunted (44.4%).

## DISCUSSION

EED is becoming relevant in public health as it is a potential cause of child stunting. This cross-sectional study was conducted among 120 children aged 36-59 months old in Quezon Province, Philippines. It aimed to determine the prevalence of stunting, STH and EED; and their relationships. EED was assessed via glucose hydrogen breath test with a cut-off of  $\geq 20$ ppm over baseline by 90 minutes suggestive of SIBO, a biomarker of EED.

Stunting remained a public health problem in the study area with a prevalence of 40%. A higher proportion of stunted children were using unimproved drinking water service level (66.7%) and unimproved toilet (80.0%). Similar patterns were observed in the study of Danaei *et al.* (2016) which highlighted that environmental factors, particularly unimproved sanitation, is attributable to 7.2 million cases of stunting worldwide. It also presented that environmental risk factors such as poor water quality, poor sanitary conditions, among others have the second largest impact on stunting globally. A cross-sectional study in Indonesia also highlighted that the chances of stunting was three times higher among children in households with untreated water and unimproved latrine (Torlesse *et al.*, 2016).

For soil transmitted helminthiasis, results showed that the prevalence of children having *Ascaris lumbricoides* was 38.1%, which is considered alarming in terms of morbidity control based on the WHO's recommended cut-off of <20% cumulative prevalence. In addition, *Trichuris trichiura* and

hookworm infections were observed in 15.3% and 1.7% children, respectively. A cross-sectional study in Argentina among school age children showed a strong association between unimproved sanitation and unimproved drinking water with the presence of STH infection (Echasu, 2017). A meta-analysis of more than 40,000 studies (Strunz, 2015) further established the link between WASH factors and the presence of STH. It was estimated that an increase of 10,000 epg egg count of *A. Lumbricoides* will increase the odds of having stunting by 47% among children and adolescents (Jardim-Botelho *et al.*, 2008). Pathogen colonisation may promote chronic inflammation and induce changes to intestinal microbiota, contributing to stunting and EED (Humphrey, 2009; Owino *et al.*, 2016; Watanabe & Petri, 2016; Gilmartin & Petri, 2016; Harper *et al.*, 2018). Among the pathophysiological changes underlying EED, the study of Harper *et al.* in 2018 found strong evidence supporting the pathway between intestinal inflammation and stunting. Hence, further analysis of pathogen-induced changes to the intestinal microbiota as a pathway leading to stunting needs to be investigated (Owino *et al.*, 2016).

In this study, no case of EED was detected. Previous studies revealed that EED characterised by elevated expired hydrogen frequently occurs in developing areas with poor sanitation and hygiene (Korpe & Petri, 2012; Prendergast & Kelly, 2016), as well as limited public health resources, in association with microbial and parasitic contamination of food and water (Owino *et al.*, 2016). Pereira *et al.* (1991) investigated SIBO in a cohort of 340 children under the age of 5 years in rural Burma (Myanmar) and detected SIBO in 27.2% children. In the study of Dos Reis *et al.* (2007) among 5 to 11 years old in Brazil, bacterial

overgrowth was diagnosed in 18 (37.5%) of the 48 slum dwelling children and in only one (2.1%) of the 48 children in the control group. The homes of these slum dwelling children did not have running water or a sewerage system compared with the control group. Lin *et al.* (2013) supported these results in assessing the relationship of faecal environmental contamination and environmental enteropathy among children living in different levels of household environmental cleanliness across rural Bangladesh.

The absence of EED in this study was probably due to a better environmental condition of the study households compared with the above mentioned literatures. Specifically, majority of the households in the study areas had access to: (1) improved drinking water (55%); (2) improved sanitation (60.8%); and (3) availability of water (99.2%) and soap (70%) for hand washing. Only 11.7% of the households had earth floor material and only 39.2% raised animals.

Several limitations can be identified in the present study. The cross-sectional design of this study did not allow any inference with regards to causal relationships. Similar to the findings of Donowitz *et al.* (2016), non-detection of EED in this study might be due to the limitation of cross-sectional analysis which only detects children with SIBO at the time of testing and may have missed children who have had recent or prolonged SIBO in their lifetime. Furthermore, the study is only a representative of one Province and the results therefore cannot be generalised for the whole population of the Philippines. Regardless of these limitations, to our knowledge, this was the first study on EED among children in the country. In addition, helminth colonisation on EED has not been well studied, thus the study was an attempt

to establish an association between STH and EED.

## CONCLUSION

The study revealed that there was no prevalence of EED detected among 36- to 59-month-old children in Quezon Province. The prevalence of STH among children were 38.1% for *Ascaris lumbricoides*, 15.3% for *Trichuris trichiura*, and 1.7% for hookworm infections. Stunting prevalence was recorded at 40%. Nutrition interventions together with the use of anthelmintic drugs, provision of clean water, environmental sanitation, and promotion of hygiene practices must be intensified to address problems on stunting and STH. This study revealed trends that warrants further epidemiological studies. The Hydrogen Breath Test employed can be adapted for a case control study with stunted subjects in areas with poor environmental conditions.

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## Authors' contributions

ARB, principal investigator, conceptualised and designed the study, conducted data collection and analysis, prepared the manuscript; CVCB, VBM and MMP reviewed and provided technical inputs on the study design, data analysis and manuscript; NAT, provided technical inputs on the study and sampling design, data analysis and interpretation, and reviewed the manuscript.

## Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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